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PHOTOPERIODIC CONTROL OF DIAPAUSE IN THE MARINE CALANOID COPEPOD *LABIDOCERA AESTIVA* [1]

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It has long been recognized that many marine copepods are seasonally present or absent in the plankton. Fish and Johnson (1937) postulated that such species probably survived as resting eggs on the sea bottom during periods unfavorable for their existence in the plankton. Resting eggs in marine bottom sediments have now been documented for several temperate neritic calanoid copepods and cladocerans (Grice and Gibson, 1975, 1977; Johnson, 1980; Kasahara *et al.*, 1974, 1975; Onbe, 1973, 1978), and it has been shown for some of these species that the maximum abundance of resting eggs in the sediments alternates seasonally with the maximum abundance of individuals in the plankton (Kasahara *et al.*, 1975). However, it has been demonstrated experimentally for only a few species that these resting eggs are in diapause rather than quiescence (Grice and Gibson, 1977; Johnson, 1980; Kasahara and Uye, 1979; Marcus, 1979; Zillioux and Gonzalez, 1972).

Diapause typically necessitates complex changes at the biochemical and physiological levels (see Clutter, 1978). In an environment that fluctuates in a predictable way, the ecological and evolutionary success of species must depend on individuals being able to accurately forecast the changes so that sufficient time is allowed for the requisite adaptive responses prior to the onset of unfavorable conditions. Marcus (1979) demonstrated that the onset of egg diapause in the marine calanoid copepod *Labidocera aestiva* precedes the decline in surface water temperatures by 2 weeks, and suggested that some factor other than temperature alone was important in triggering the onset of dormancy in this species. Data presented in this paper demonstrate that photoperiod significantly affects the induction of diapause in *L. aestiva*.

Materials and Methods

Rearing of nauplii, copepodites, and adults

Labidocera aestiva were reared from the first naupliar stage through to reproductive maturity in 19 l glass carboys containing 5-μm filtered sea water. The carboys were mounted at an angle on a frame which rotated at 2 rpm. This rotation served to keep the algal food (see below) in suspension. The entire apparatus was in a walk-in incubator equipped with temperature controls and a 24-hr light : dark cycle, with fluorescent lights providing illumination of 200–300 ft-candles. All developmental stages were fed a mixture of *Gymnodinium nelsoni*, *Gonyaulax polyedra*, *Prorocentrum micans*, and *Peridinium trochoideum*. Dinoflagellates were obtained from Robert Guillard, Woods Hole Oceanographic Institution, Woods Hole, MA, and were subsequently cultured in Fernbach flasks containing Guillard's f/2 media (Guillard, 1972) at 17°C and 12L : 12D cycle.

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Before the copepods reached reproductive maturity, the contents of each carboy were siphoned weekly through a 63 μm Nitex screen to collect surviving copepods. The vessels were washed, refilled with fresh filtered seawater, and supplied with food. The dinoflagellates were added in equal numerical concentrations to give a final density in the carboy of 7.0–10.0 × 10^2 cells/ml. Surviving copepods were returned to each carboy. When egg production began, the number of males and females in each carboy was equalized to a density of 25–30 individuals of each sex by removing adults at random with a wide-mouth pipette from the filtrate. Subsequently the carboys were siphoned every 2–3 days so that eggs could be collected before they hatched. Eggs were removed from the filtrate (retained by the screen) with a micropipette and placed in 5-μm filtered sea water. Adults were returned to the carboy. The weekly schedule for changing the sea water and food remained the same.

Experimental procedures

Carboy populations were initiated with 200 nauplii derived from subitaneous eggs (carboys 7, 8, 9, 12, 14, 15) or chilled resting eggs (carboys 4 and 6) produced either in the laboratory (first generation) or by freshly collected field females. Copepods were reared at 13.5°–15.5°C in five carboys exposed to a short-day photoperiod (8L : 16D), and three carboys exposed to a long-day photoperiod (18L : 6D). Eggs were collected from each carboy every 2–3 days for 8–10 days. At each collection the total numbers of eggs and surviving males and females were determined, and a sample of 100–120 eggs (from each collection day) was placed in glass-fiber-filtered sea water and incubated at 25°C and 12L : 12D to hasten the time to hatching. After 4–5 days the percent hatch was determined, and unhatched eggs were placed in two jars (50 eggs/jar) at each of 5° and 25°C. The eggs were kept at these temperatures a minimum of 40 days. At the end of this interval the 5°C eggs were warmed at 25°C and the percent hatch ascertained. At the same time, the hatch of eggs incubated at 25°C was also determined. In addition, a minimum of 20 adult males and females from each carboy were preserved in 5% buffered formalin and subsequently measured with a stereomicroscope to determine cephalothorax and total body length. The body sizes of these copepods and adults collected in the field under similar temperature conditions were compared, to assess the suitability of growth conditions in the laboratory.

Results

The age at reproductive maturity of *Labidocera aestiva* reared in the laboratory at 13.5°–15.5°C ranged from 27.2 ± 1.3 days at 8L : 16D to 22.3 ± 1.5 days at 18L : 6D. The survival of individuals to reproductive adulthood was good (65–75%) for each experimental carboy. Copepods reared under the long-day regime of 18L : 6D produced more eggs during the experimental period (800–1000 eggs in each carboy per day) than the individuals reared under the short-day regime of 8L : 16D (227–580 eggs in each carboy per day).

Table I shows the percent hatch data for one set of eggs for each collection day for carboy 6, and is representative of results for the other egg set as well as the other carboys. For carboy 6, final hatch at 25°C was high (> 90%) after chilling at 5°C for incubation periods ranging from 49 to 123 days. The hatch of eggs kept at 25°C appears to increase (26–43%) as the incubation period

TABLE I

Percent hatch of eggs produced by females reared at 13.5°–15.5°C and 8L:16D in Carboy 6. Eggs that did not hatch within 4–5 days at 25°C (initial) were incubated in jars at 5° or 25°C. The final hatch of these eggs at 25°C is indicated, as well as the period of incubation.

Date collected	Initial hatch at 25°C	Incubation (days)	Final hatch	
			5°C	25°C
3 August 1979	4%	49	95%	26%
6 August 1979	1%	62	97%	36%
8 August 1979	7%	81	90%	39%
10 August 1979	2%	99	93%	37%
13 August 1979	7%	123	91%	43%

increases. The percent hatch of eggs and adult body lengths for each carboy is shown in Table II. The values for percent initial hatch for each carboy represent an average of values determined each time the eggs were collected. Similarly, the values for percent final hatch for each carboy after incubation at 5° and 25°C represent an average of values derived from the unhatched eggs subsequently incubated from each collection day.

Subitaneous (quickly hatching) and diapause egg production were strikingly different between the two photoperiod regimes. At 8L:16D, 13.2 ± 4.3% of the eggs produced hatched within 4–5 days (classified as subitaneous), whereas at 18L:6D, 89.9 ± 1.4% of the eggs were subitaneous. The similarity of subitaneous egg production among replicates was slightly less at 8L:16D than at 18L:6D, ranging from 2.9 ± 0.8% to 23.8% ± 1.9, and 87.3 ± 2.5% to 92.0 ± 1.7%, respectively. Eggs produced at 18L:6D that did not hatch within 4–5 days appeared brown and granular. They were dead eggs (Marcus, unpublished), and were not incubated at 5° or 25°C. Most of the unhatched eggs produced at 8L:16D were green and had a clear perimeter. These eggs were incubated at 5° and 25°C and, as shown in Table II, an average of 85.9 ± 3.1% hatched after chilling and were classified as diapause eggs. The remainder, which did not hatch, were dead. Some of the eggs (38.1 ± 5.3%) kept at 25°C did hatch during the incubation interval.

The difference in body lengths of adult males and females reared in replicate carboys for a given photoperiod regime was small. The widest range of values resulted at 8L:16D for male total length (2.10 ± 0.11 mm to 2.24 ± 0.13 mm). For each experimental regime females were longer than males (both total length and cephalothorax). Moreover, individuals reared under the short-day regime (8L:16D) were larger than individuals reared at 18L:6D.

DISCUSSION

The results presented in this paper provide the first evidence of the importance of photoperiod as a trigger for the induction of diapause in a marine copepod, although this effect has been shown for several fresh-water copepods and cladocerans (Stross and Hill, 1965; Stross, 1969a, b; Watson and Smallman, 1971; Bunner and Halcrow, 1977). Copepods which developed at 13.5°–15.5°C under 18L:6D produced 89.9 ± 1.4% subitaneous eggs, whereas individuals reared at 8L:16D produced only 13.2 ± 4.3% subitaneous eggs. Moreover, 85.9 ± 3.1% of the non-subitaneous eggs produced at 8L:16D hatched synchronously at 25°C after

TABLE II

Percent hatch of eggs produced by females reared at 13.5°–15.5°C under 8L:16D or 18L:6D. Eggs that did not hatch within 4–5 days at 25°C (initial) were incubated in jars at 5° and 25°C. The final hatch of these eggs at 25°C is indicated, as well as the cephalothorax (CT) and total body (TL) lengths in millimeters attained by adult males and females for each carboy. $\bar{X}$ = mean.

Photoperiod	Carboy #	Percent hatch (±SE)			Size (±SD)			
		Initial hatch at 25°C	Final hatch		♀		♂	
			5°C	25°C	CT	TL	CT	TL
8L:16D	4	13.1 ± 3.3	82.4 ± 5.8	23.0 ± 5.2	1.74 ± 0.07	2.37 ± 0.09	1.72 ± 0.07	2.24 ± 0.13
	6	4.2 ± 1.2	92.2 ± 1.1	34.7 ± 3.4	1.80 ± 0.07	2.40 ± 0.10	1.70 ± 0.05	2.23 ± 0.08
	7	22.0 ± 4.1	89.3 ± 1.8	34.1 ± 4.1	1.78 ± 0.08	2.37 ± 0.09	1.71 ± 0.09	2.22 ± 0.10
	8	2.9 ± 0.8	89.9 ± 1.8	44.4 ± 6.4	1.70 ± 0.05	2.26 ± 0.08	1.65 ± 0.06	2.14 ± 0.08
	9	23.8 ± 1.9	75.5 ± 5.1	54.4 ± 5.77	1.72 ± 0.06	2.28 ± 0.08	1.61 ± 0.07	2.10 ± 0.11
$\bar{X}$		13.2 ± 4.3	85.9 ± 3.1	38.1 ± 5.3	1.75 ± 0.04	2.23 ± 0.06	1.68 ± 0.05	2.19 ± 0.06
18L:16D	12	87.3 ± 2.5	—	—	1.66 ± 0.08	2.21 ± 0.12	1.55 ± 0.08	2.03 ± 0.10
	14	90.4 ± 1.8	—	—	1.66 ± 0.05	2.21 ± 0.09	1.57 ± 0.05	2.07 ± 0.06
	15	92.0 ± 1.7	—	—	1.70 ± 0.06	2.24 ± 0.07	1.58 ± 0.06	2.08 ± 0.06
$\bar{X}$		89.9 ± 1.4			1.67 ± 0.02	2.22 ± 0.02	1.57 ± 0.02	2.06 ± 0.03

chilling at 5°C. However, chilling was not an absolute requirement to induce hatching, since some eggs kept at 25°C for a prolonged period did hatch (Tables I, II). The hatching response of diapause eggs produced in the laboratory under a short-day regime is similar to the pattern demonstrated for diapause eggs produced by field-collected females (Marcus, 1979). Copepods reared in the laboratory under a long-day regime did not produce diapause eggs.

The genetic composition of a female ultimately restricts the potential range of egg types she can produce, but the egg type actually realized is determined by the environmental conditions she experiences during development. This is evident from the production of diapause eggs at 8L : 16D by females which developed from both diapause eggs (carboys 4 and 6) and subitaneous eggs (carboys 7, 8, and 9). Similarly, subitaneous eggs were produced at 18L : 6D by females which developed from subitaneous eggs (carboys 12, 14, and 15).

The data on body sizes of *L. aestiva* reported herein provide a valid estimate (see Paffenhöffer, 1970) of the suitability of the laboratory conditions tested in this study. The total lengths and cephalothorax lengths attained by adult males and females reared at 13.5°–15.5°C under both long- and short-day regimes (Table II) are comparable to the sizes of field adults collected at a similar water temperature in Vineyard Sound, MA (Marcus, 1979). The fact that individuals reared at 18L : 6D were smaller than individuals reared at 8L : 16D was probably due to temperature. The incubator in which the copepods were reared undergoes a slight temperature change as a result of the light cycle. When the lights were on, the air temperature in the incubator was maintained at 15.5°C, but with the lights off the temperature dropped to 13.5°C. Presumably the water in the carboys also experienced this temperature shift, although to a lesser extent. Thus, the copepods reared under the 18L : 6D regime experience slightly warmer temperatures overall (average 15.0°C/L : D cycle), than individuals reared at 8L : 16D (average 14.2°C/L : D cycle). Based upon the inverse relationship between body size and temperature which has been documented for *Labidocera aestiva* collected in the field (Marcus, 1979), the differences observed for the laboratory-reared copepods can perhaps be accounted for by the slightly different thermal regimes.

An alternative explanation, based on feeding patterns, could also account for the observed differences. If *L. aestiva* feeds primarily at night, it is possible that under the short-day regime (8L : 16D) individuals were able to consume more food and therefore attained a larger size. If this were the case, however, it might be expected that greater food consumption would result in an increase in the number of eggs produced. These results were not obtained. In fact, copepods reared at 8L : 16D produced fewer eggs. It may be that a diapause egg is able to overwinter because it contains more storage nutrients than a subitaneous egg. This could result in the production of fewer diapause eggs to balance the increased energy requirement. Without more information on *L. aestiva* feeding pattern and egg biochemistry, this problem cannot be clarified. Nevertheless, sizes attained by laboratory animals suggest that conditions used to rear *L. aestiva* in the laboratory adequately simulated the conditions for good development, growth, and reproduction experienced by this species in the field. Therefore, it is assumed that the factors which were shown to stimulate the production of diapause and subitaneous eggs in the laboratory reflect a similar function in the field.

The influence of photoperiod on the life history of *L. aestiva*, as suggested by this study, correlates well with the annual life cycle of this species in Vineyard Sound, MA. (Fig. 1). *L. aestiva* adults first appear in the plankton in early

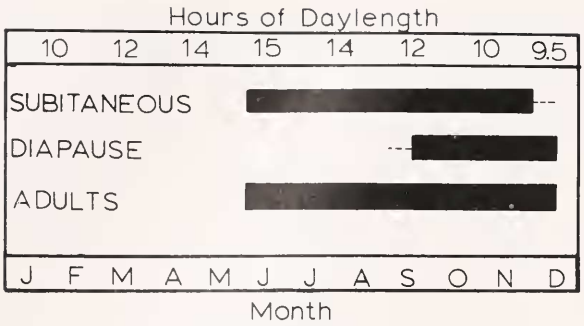

FIGURE 1. Schematic diagram of seasonal occurrence of adults and production of subitaneous and diapause eggs of *Labidocera aestiva* in Vineyard Sound, MA, with respect to daylength.

summer when surface water temperatures have reached 18°–20°C (Marcus, 1979) and the photoperiod is 15L:9D (U. S. Dept. of Commerce, 1979). In August, daylength is somewhat shorter (14L), and water temperature is maximal (22°–23°C). By mid-September, surface water temperature has declined to 19°C and photoperiod is 12L:12D. Temperatures drop to 15°C by mid-November, and daylength (9 3/4L) is further reduced. In mid-December when nauplii, copepodites, and adults disappear from the plankton, surface water temperature has dropped to 6°C, and daylength is minimal at 9 1/2L:14 1/2D. *Labidocera aestiva* produces both subitaneous and diapause eggs in November in Vineyard Sound (Marcus, 1979). Similarly, *L. aestiva* reared in the laboratory under the regime (13.5°–15.5°C, 8L:16D) that approximates Vineyard Sound in November produce both subitaneous and diapause eggs. Moreover, the majority of eggs produced by this combination of photoperiod and temperature in both the field and laboratory are diapause eggs. The same combination of factors again prevail in Vineyard Sound in mid-March, but at this time *L. aestiva* adults are not present. When they do reappear in early summer the photoperiod is approximately 15L:9D, and only subitaneous eggs are produced. The results for the alternate regime (13.5°–15.5°C, 18L:6D) tested in this study, while not exactly identical to the field situation experienced by *L. aestiva*, nevertheless suggest that under long-day photoperiods, this species produces subitaneous eggs. The results do not imply that photoperiod is the only factor influencing the production of diapause eggs by *L. aestiva*, nor that it is of primary importance. Other factors (*e.g.*, temperature, density, food) may have a similar function and/or may modify the effects of photoperiod, as has been shown for insects (Saunders, 1976), freshwater copepods (Watson and Smallman, 1971), and cladocerans (Bunner and Halcrow, 1977; Stross, 1969b; Stross and Hill, 1965). Thus, it is possible that changes in the quantity or quality of the food supply during the weekly feeding interval may have influenced the type of eggs produced by *L. aestiva* in this study. Most recently, it has been suggested that temperatures below 15°C induce the production of dormant eggs by the estuarine calanoid copepod, *Acartia californiensis* (Johnson, 1980). Gaining insight into the factors which control diapause of marine copepods is fundamental to elucidating the mechanism that regulates their seasonal occurrence in the plankton.

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Summary

The calanoid copepod *Labidocera aestiva* was reared in the laboratory at 15°C. Individuals that developed under a photoperiod regime of 18L:6D produced subitaneous eggs, whereas copepods exposed to a short-day regime of 8L:16D produced mostly diapause eggs. The results indicate that photoperiod is an important factor controlling the life cycle of *L. aestiva*. It is suggested that in Vineyard Sound, MA, this species produces subitaneous eggs during the summer in response to long daylengths, and in the fall produces mostly diapause eggs in response to short daylengths.

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